



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C07K 14/715 // 1/30</b>	A1	(11) International Publication Number: <b>WO 98/35991</b> (43) International Publication Date: 20 August 1998 (20.08.98)
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(21) International Application Number: PCT/SE98/00277 (22) International Filing Date: 17 February 1998 (17.02.98)  (30) Priority Data: 9700566-4 18 February 1997 (18.02.97) SE  (71) Applicant (for all designated States except US): PHARMACIA & UPJOHN AB [SE/SE]; S-112 87 Stockholm (SE).  (72) Inventors; and (75) Inventors/Applicants (for US only): SUNDSTRÖM, Michael [SE/SE]; Kommendörsgatan 12, S-114 48 Stockholm (SE). NORSTEDT, Gunnar [SE/SE]; Författarvägen 46, S-161 42 Bromma (SE).  (74) Agents: FORSLUND, Niklas et al.; Pharmacia & Upjohn AB, S-112 87 Stockholm (SE).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
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(54) Title: MODIFIED CYTOKINE RECEPTOR PROTEIN

## (57) Abstract

Disclosed is a modified extracellular domain of a cytokine receptor protein, capable of being crystallized without being complexed to a ligand molecule. The receptor preferably is a homo- or heterodimeric cytokine receptor, having at least one molecule segment which contributes to a disordered structure deleted. The most preferred receptor is human growth hormone receptor (hGHR). Also disclosed are crystals of unliganded modified receptor suitable for binding studies with ligand candidates, a method of obtaining the crystals, as well as a method of designing drugs with cytokine receptor activity by employing such crystals according in binding studies with selected ligand candidates.

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## MODIFIED CYTOKINE RECEPTOR PROTEIN

**Field of invention**

5        The present invention relates to modified extracellular domains of cytokine receptor proteins which are capable of being crystallized without being complexed to a ligand molecule, methods of preparing such proteins and crystals formed of the modified proteins.

10      **Background of the invention**

Human growth hormone (hGH) is a key factor in the regulation of certain physiological processes, such as growth and differentiation of muscle and bone cells. The hGH signal is mediated by homodimerization of two identical human growth 15 hormone receptors (hGHR)<sup>1</sup>. To accomplish this task, hGH interacts with each receptor using two distinct binding epitopes (hGH site 1 and 2), that both bind at the domain interface of the extracellular part of the receptor<sup>2</sup> (Fig. 1). However, an important and hitherto unresolved question is if the receptor undergoes conformational changes to facilitate hGH binding. hGH binds to the hGHR but also to 20 its naturally occurring soluble binding protein (hGHbp)<sup>3</sup>. Heterologously expressed variants of hGHbp are therefore commonly used to study the extracellular events in receptor dimerization<sup>4</sup>. Moreover, in a zinc dependent interaction, hGH also can bind to the human prolactin receptor (hPRLR)<sup>5</sup>.

It would be highly desirable to obtain a model to study the structural 25 requirements for the transition of the extracellular part of the human growth hormone receptor from its free, unliganded state to its homodimeric state complexed with a ligand. Such studies could potentially lead to a more detailed understanding of the binding conditions specific for hGHR and would constitute important means in obtaining new drug candidates with ligand activity. However, it has so far been

impossible to perform binding studies of hGHR in crystalline form without having the receptor molecule bound to a natural ligand. The reason is that both hGHR and other cytokine receptors have been found difficult to crystallize in their unliganded form, since they most likely contain domains and/or loop regions that are flexibly connected  
5 which contributes to a disordered state which obstructs crystallization.

It is the object of the present invention to provide modified extracellular domains of cytokine receptors which solve the described problems and which are capable of being crystallized with conventional methods. Furthermore, it is an object of the present invention to provide crystals of modified extracellular receptor proteins  
10 which are exceptionally useful for binding studies with small molecules that, in the absence of the natural ligand, are free to interact with the receptor binding surfaces.

### Description of the invention

15 According to a first aspect, the present invention is directed to a modified extracellular domain of a cytokine receptor protein, capable of being crystallized without being complexed to a ligand molecule. These modified proteins substantially maintain their activity to their native ligands and they will therefore constitute powerful tools for ligand interaction studies. The inventive, modified cytokine  
20 receptor preferably is of the type which oligomerizes when being bound to a ligand. This may include heterooligomerization or homodimerization, as discussed in Mol. Cell. Biol., 1994, Vol. 14(6), p.3535-49: S Watowich et al. Most preferably, the modified receptor is a homodimeric cytokine receptor, such as the growth hormone receptor (hGHR) having an extracellular part consisting of 237 amino acids in its  
25 native state. The inventive proteins have at least one molecule segment contributing to a disordered structure deleted. Preferably the deletion results in a truncation in at least one terminal end and most preferably it is truncated both in its C-terminal end and in its N-terminal end. More preferably, the inventive proteins are modified human growth hormone receptors (hGHR) with 31 or 33 amino acid residues removed in its  
30 N-terminal end and/or with 3 or 4 amino acid residues removed in its C-terminal end.

Even more preferably, the inventive modified human growth hormone receptor (hGHR) consists of the amino acid residues 32-237, 32-234 or 34-233 of the native molecule. Of these modified molecules, the truncated receptor consisting of amino acids 32-234 of the native molecule is the most preferred. It should be emphasized 5 that said modified cytokine receptors would be readily produced by the skilled person with existing methods of recombinant technology and their production in a recombinant host and their subsequent purification, therefore are not parts of the present invention.

According to another aspect, the present invention relates to crystals of 10 unliganded modified cytokine receptor which are surprisingly suitable for binding studies with ligand candidates and can readily be produced according to conventional crystallization technology. The inventive crystals can be formed at a suitably favorable pH interval and in the presence of a great variety of salts in suitable concentration range. In addition, the crystals have surprisingly favorable 15 characteristics for ligand binding studies. Such an important characteristics are the extension of the crystal packaging surfaces or the contact area between the molecules of the crystal, the solvent uptake capacity, resistance to conventionally employed solvents and their capacity of being frozen with maintained capacity of diffracting during X-ray investigation.

20 The crystals of the inventive modified cytokine receptors can contain more than 50 % (v/v) of a solvent acceptable for binding studies, preferably more than 60 % (v/v) of a solvent and even more preferably between 60 to 80 % (v/v).

The contact surface between two molecules in the crystal lattice, i.e. the 25 surface buried from solvent, related either by crystallographic or non-crystallographic symmetry, should suitably be in the range 200-1800 Å<sup>2</sup>. Thus, 100-900 Å<sup>2</sup> on each molecule at crystal packaging interfaces should be made solvent inaccessible at contacts with other molecules in the crystal lattice.

Accordingly, the crystals according to the present invention preferably have a contact 30 surface between two molecules of between 200 to 1800 Å<sup>2</sup> (square ångström) and more preferably between 100 to 900 Å<sup>2</sup> (square ångström), as determined by surface area calculation performed with the program DSSP (Kabsch & Sander 1983).

The crystals according to the present invention are possible to freeze using nitrogen in liquid or gas form and maintain their capacity of diffracting to at least 3.5 Å and preferably to at least 2.3 Å, or better, when using synchrotron radiation source.

Furthermore, the inventive crystals have an excellent resistance to solvents conventionally used in ligand binding studies. The crystals are resistant to an addition of about 10 % (v/v) of dimethylsulfoxide (DMSO) or 5 % (v/v) of dimethylfluoride (DMF) for at least 24 hours in a crystallization droplet.

A further aspect of the present invention is a method of designing drugs capable of activating cytokine receptor signal transduction activity by employing said inventive crystals in binding studies with selected ligand candidates. In the method, such crystals are preferably soaked with a solution comprising the ligand or co-crystallized with the ligand. The methods may include oligomerization of the receptor induced by specific ligand attraction, such as dimerization of the receptor, when, for example, crystals of a modified growth hormone receptor is investigated with ligands with potential growth hormone activity.

A yet further important aspect of the invention is the provision of a method of obtaining improved cytokine receptor crystals involving the subsequent steps of solving the receptor three-dimensional structure complexed to a ligand by crystallographic methods, identifying regions of the receptor molecule which may contribute to disorder in a crystalline state, producing modified receptor molecules without said regions, and crystallizing the modified receptor without the presence of a ligand. Preferably, in this method, the extracellular part of the receptor has a known primary amino acid sequence and preferably, it is studied when bound to its ligand by conventional crystallographic methods involving such steps as crystallization and X-ray structure determination. Further, such modified proteins can also be used in structural determinations using other techniques such as Nuclear Magnetic Resonance (NMR). From these results it is conceivable to identify domains in the receptor molecule which will contribute to a disordered state in crystalline form and subsequently to produce them deprived of such domains with conventional methods.

This method has proved especially successful for human growth hormone receptor initially crystallized with its native ligand human growth hormone.

**Detailed and exemplifying description of the invention**

Fig. 1 schematically illustrates the general features of the hGH:hGHR 1:2 complex. A single hormone molecule binds two hGHR molecules at the interface of the two domains. 2700 Å<sup>2</sup> becomes solvent inaccessible In hGH site 1 - receptor interactions, whereas only 1300 Å<sup>2</sup> are covered in site 2 interactions. An additional surface area of 900 Å<sup>2</sup> is buried in receptor - receptor interactions. of the receptor needed for hormone interactions, 104 and 169, are depicted in white. This picture was generated using a 2.5 Å structure of the 1:2 complex<sup>6</sup>. All figures were produced using the programs MOLSCRIPT<sup>27</sup>, RASTER3D<sup>28</sup> and RENDER<sup>29</sup>.

Fig. 2 schematically demonstrates a comparison of the crystal structures of free hGHR and hGHR interacting with hGH site 1 in the 1.2 complex. In the structural alignment, only the C-terminal domains were used (residues 128 - 234) in order to clearly visualize the domain - domain movements upon transitions from free to complexed form, a) side view b) front view. In the free receptor crystal the two hGHR molecules in the asymmetric unit are very similar with a root mean square deviation of 0.18 Å comparing 184 Ca positions. The corresponding values for the liganded receptors in the 1:2 complex are 1.04 Å (181 Ca positions) and 1.17 Å (172 Ca positions) for the hGH site 1 and site 2 binding receptor respectively.

Fig 3. is a comparison of the conformation of tryptophane residues 104 and 169 as well as adjacent loops. The conformations of Trp169 are virtually identical in both the liganded variants of the receptor. In contrast, Trp104 show distinct conformations and thus is to a greater extent conformationally adapted upon hGH interactions, facilitating high affinity ligand binding. The favorable interdomain hydrogen bond (Glu44 - Gln166) only seen in the hGH site 2 binding receptor, is enabled by main and side chain conformational alterations of the loop 163 - 168.

Thus, this loop adaptation may be necessary to allow site 2 interactions to be formed and stabilized.

According to the present invention, new modified hGH binding molecules were engineered to improve crystal forming properties by N- and C-terminal truncations and used to resolve this question at the structural level. The present invention provides the first three dimensional structure of an unliganded hematopoietic receptor determined at 2.3 Å resolution. By putting the present invention into practice, significant structural differences, both in domain orientations and side chain conformations compared to hGH bound receptors are observed, as disclosed below in more detail.

Initially, with the purpose to understand the structural requirements for receptor transition, from unliganded monomeric state to homodimerized, investigations to determine the native free receptor structure. However, despite strenuous efforts all attempts failed to crystallize the native molecule, hGHR1-237 (consisting of residues 1 - 237 of the hGHR extracellular domain). In order to provide modified receptor molecules more suitable for structural studies, the 2.5 Å resolution structure of the hGH:hGHR 1:2 complex<sup>6</sup> was initially studied. It was found that the first 31 residues at the N-terminus of hGHR could be disordered in the crystal structure of both receptor molecules in the 1:2 complex. In addition, this region is susceptible to proteolytic degradation. It was therefore attempted to truncate this N-terminal domain of the region and perform and subject the modified molecules to crystallization. In surprising contrast, the truncated hGHR32-237 crystallized readily, although not of diffraction quality. A second generation of molecules were C-terminally truncated, deleting additional residues disordered in the 1:2 complex and thereby also potentially contributing to the disorder of the free receptor molecule. One such receptor variant, hGHR32-234 not only yielded crystals of surprisingly good diffraction quality (Table 1) but also displayed surprisingly improved properties with regard to expression levels, solubility and stability during the purification process.

Moreover, the N-terminal truncation yields a molecule similar in sequence to the naturally occurring hGHR exon-3 splice variant<sup>7,8</sup>. It was also confirmed that the truncated hGHR variants bind hGH with the same stoichiometry and very similar affinity as the native molecule.

5       The crystals obtained contain more than 60% (v/v) solvent and have only minor interactions with neighboring molecules in the crystal lattice. Thus, the unliganded receptor structure is highly suitable for structural comparisons. In addition, crystal soaking experiments are highly feasible, since spacious solvent channels within the crystals will allow diffusion of small hGH mimicking ligand molecules to  
10      the receptor.

The modified receptors according to the present invention have enabled, for the first time, detailed comparisons of inactive or unliganded receptors in comparison with the active or liganded form. By means of the present invention, several new findings therefore are presented below which clearly demonstrate its considerable  
15      importance for understanding the molecular mechanisms of receptor activation. In particular, it is crucial to be provided with a structural basis for receptor activation.

The two extracellular domains of the receptor have topologies similar to the fibronectin type III fold<sup>2</sup>. In the 1:2 complex of hGH with hGHR, the two receptor molecules are very similar to each other. In the crystal structure of the 1:1 complex of  
20      an hGH antagonist mutant (where Gly120 has been substituted with an arginine - hGH-G120R), with hGHR, the structure of the receptor is remarkably similar to the corresponding molecule in the 1:2 complex<sup>6</sup>. These structural studies suggest that no significant conformational changes of the hGH site 1 binding receptor occurs upon binding of the second receptor. Although the 1:2 complex structure revealed that it is  
25      possible that binding of hGH could influence the domain orientation of the receptor<sup>2</sup>, it has been suggested that no large conformational adaptations of either hGH or hGHR would occur when the ternary complex is formed<sup>9</sup>.

It was also observed that the domain orientation differed significantly, when comparing the structures of liganded and unliganded hGHR. When a structural alignment of the C-terminal domains (residues 128 - 234) is made, a large shift in the N-terminal domain (residues 32 - 123) between ligand bound and free receptor form is 5 observed. The angle between the two domains is considerably higher (7°) in the free receptor form than for the receptor molecules in the 1:2 and 1:1 complexes. Thus, the corresponding atom positions are translated 4 Å vertically and 2.5 Å horizontally, comparing residues furthest away from the interdomain connecting loop (Fig. 2).

Trp104 and Trp169 in the receptor have been identified as the key residues in 10 hormone binding<sup>10</sup>. Since hGH can interact with the receptor using two distinct binding sites, the loop containing Trp104 occupies two different conformations to enable receptor homodimerisation<sup>2</sup>. In the unliganded receptor, the conformations of main and side chains in the loop containing Trp104 are similar to the conformation observed in the receptor binding to hGH site 2 (Fig. 3). Therefore, the hGH site 1 15 interactions to a greater extent compared to hGH site 2, induce an conformational adaptation of the Trp104 side chain and its loop region. In contrast, the conformation of Trp169 is very similar in the free and the liganded receptor molecules (Fig. 3). The structural rigidity of Trp169 could suggest a role for this residue in the initial hormone - receptor recognition event. However, the loop preceding Trp169 (residues 163 - 20 168), is conformationally adapted upon hGH site 2 interactions but is very similar in the liganded hGH site 1 binding and the unliganded receptor. The structural adaptations of the two loop regions (102 - 106 and 163 - 168) in the receptor seem to enable binding to hGH binding site 1 and 2, respectively.

In the unliganded receptor, favorable interdomain interactions can be observed 25 between Arg39 NH1 - Gln130 O (2.4 Å), Arg39 NH2 - Asp132 Od1 (3.2 Å), Ser40 N - Ile128 O (2.8 Å), Arg43 NH1 - Trp169 O (2.7 Å) and Arg43 NH2 - Trp169 Ne1 (3.2 Å). Another key residue, Asp126, contributes indirectly by stabilizing the conformation of Arg43. Essentially the same interactions are observed in both

liganded receptors in the 1:2 complex, but with some differences in apparent bond lengths. An additional interdomain interaction, Glu44 Oe1 - Gln166 Ne2 (2.8 Å), is observed only in the hGH site 2 binding receptor and is most likely facilitated by structural adaptation of the loop 163 - 168. Hence, the observed differences in domain orientations between liganded and unliganded receptors seem to be only to a minor extent caused by forming and breaking of interdomain hydrogen bond interactions. Since a certain degree of domain flexibility appears to be an inherent property of the extracellular part of hGHR, fine tuned adjustments in interdomain interactions could contribute significantly to the domain rearrangement upon ligand binding. In this context, it is important to stress the fact that virtually no crystal packing interactions from adjacent molecules in the crystal lattice can be observed in regions predicted to be essential for domain - domain interactions or hGH binding in the present study.

No structure of native hGH in its unliganded form is available, but the crystal structure of a hGH site 1 high affinity mutant showed that the structure of the hormone in the absence of receptor interactions was similar to the receptor bound native molecule<sup>11</sup>. In addition, in the 1:1 complex we could observe that the N-terminus (residues 1 -6) of hGH-G120R occupies a significantly different conformation in the absence of the receptor interactions and thus is conformationally adapted upon formation of the ternary complex. Other differences between the hormone molecules in the 1:1 and the 1:2 complex are minor<sup>6</sup>.

Another cytokine receptor, the erythropoietin receptor (EPOR) is constitutively active when disulfide-linked homodimers are formed in the extracellular domain<sup>12</sup>. It has also been shown that cyclic peptides derived from phage display screening could dimerize and activate the EPOR<sup>13,14</sup> as well as can association of the EPOR with a virus glycoprotein<sup>15</sup>. Since no similar mechanisms have been reported for hGHR, the mode of EPOR activation appears promiscuous compared to growth hormone receptors. The PRLR<sup>16,17</sup> and EPOR<sup>13</sup> structures revealed significantly different domain orientations of the receptors compared to the hGHR. Since the PRLR

interact with both prolactin and hGH, structural adaptations of the receptor molecule could facilitate binding of these two related but distinct hormone ligands.

Structural adaptations of hGHR upon ligand binding could serve several functions. Obviously one is to provide the specificity and affinity needed for the interaction. Another possibility is that the interdomain rearrangement enables the formation of disulfide linked receptor dimers upon hGH exposure<sup>18</sup>. Ligand induced hGHR conformational adaptation could thus facilitate receptor dimerization and be an important mechanism to keep unliganded receptors inactive. Since dimerization of cell surface receptors most likely is a general mechanism to initiate intracellular signal transduction<sup>19</sup>, conformational adaptation of receptor molecules upon ligand binding, as observed in this study, may be applicable to other systems as well. The invention as described in the appended claims, therefore should be regarded as generalizable beyond the claim scope.

15     Example 1

hGH and hGHR used in the protein crystallographic work were expressed and purified as previously described<sup>6</sup>. Truncation mutants of hGHR were created using standard sub-cloning techniques and the expressed protein was assayed for hGH binding using affinity and size exclusion gel filtration chromatography as well as BIACore (Pharmacia Biosensor, Sweden) measurements. The hGHR32-234 protein was crystallized by vapor diffusion. 3 ml protein solution (7 mg/ml in 10 mM ammonium acetate) was mixed with 3 ml of 0.33 M NH<sub>4</sub>SO<sub>4</sub>, 30% (w/v) PEG-2000-dimethyl ether, 1% (v/v) DMSO and 100 mM MES buffer at pH 6.4 in a sealed tissue culture 24-well plate (Falcon, USA). The crystallization droplets were equilibrated at +18°C with 1 ml of the mother liquor for 2-4 weeks to obtain optimal quality crystals that diffracted to at least 2.9 Å with a conventional X-ray source. The crystals could be frozen directly in the N<sub>2</sub> beam by adding a 1:1 mixture of 25% (v/v) ethylene

glycol and 25% glycerol (v/v) to the crystallization droplet. Data was collected at station A1 at Cornell High Energy Synchrotron Source using a CCD detector (Area Detector Systems Corp., USA). The data was indexed, processed and scaled in the tetragonal spacegroup I4 using the programs DENZO and SCALEPACK<sup>20</sup>. A molecular replacement search procedure was performed using the program AMORE<sup>21</sup>. As search molecule the co-ordinates of the site 1 binding hGHbp molecule in our 2.5 Å hGH:hGHR 1:2 complex was used. The highest scoring solution in the resolution interval 8 - 4 Å was found in space group I4 with two hGHbp molecules in the asymmetric unit. A rigid-body refinement in X-plor<sup>22</sup> with individual hGHbp domains including data between 10 - 6, 10 - 5 and 10 - 3.5 Å in each respective cycle, decreased both the R- and Free-R<sup>23</sup> value dramatically compared to previous runs where the native hGHbp domain arrangement had been used. A cyclic process of model building in O<sup>24</sup> followed by NCS restrained POWELL minimization in X-plor using data between 15 - 2.3 Å corrected for most main and side chain changes to the search molecule. At this stage the first simulated annealing run<sup>25</sup> was performed using a slow-cooling protocol from 3000 K to 300K in 50 ps steps. Solvent molecules were introduced into FoFc densities above 3.0 s. After 3 cycles, 327 solvent molecules had been introduced and assigned to the protein chain using the programs DISTANG and WATERTIDY in the CCP4 program package<sup>26</sup>. A final POWELL minimization followed by a simulated annealing run from 2500 K to 300 K in 50 ps steps including data between 15 to 2.3 Å was performed. Individual B-value refinement was added as the final step and solvent molecules with high temperature factors ( $> 50 \text{ \AA}^2$ ) or absent 2FoFc electron densities at 1.0 s cut-off were removed. The Free R-value was used to validate the progress of the entire refinement. The final model consists of residues 32 - 52, 63 - 70 and 80 - 234 of both molecules in the asymmetric unit as well as 261 solvent molecules and two sulphate ions. At the present stage of refinement the R-factor of the model is 21.7 % (R-free 29.3 %) using data between 10 - 2.3 Å. As a control, a dataset to 3.2 Å at

room temperature was collected. No significant differences to the 2.3 Å structure were observed showing that conformational adaptation was not induced by the transfer to cryogenic conditions.

Table 1.

## Crystallographic data for hGHR32-234

5	No of crystals:	1
	Resolution:	2.3 Å
	Completeness	89.7% (18-2.3 Å) 87.1% (2.4-2.3 Å)
	Multiplicity:	4.8
10	Rmerge	6.7% (18 - 2.3 Å) 24.6% (2.4 - 2.3 Å)
	Cell	104.8 104.8 115.7 Å 90° 90° 90°
	Space group	I4
15	No of solvent molecules	261
	Unique reflections	24987
	r.m.s bond deviations (Å)	0.011
	r.m.s angle deviations (°)	1.76
	model R-factor/R-free	21.7/29.3%

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**Claims**

1. A modified extracellular domain of a cytokine receptor protein, capable of being  
5 crystallized without being complexed to a ligand molecule.
2. A modified protein according to claim 1 being a homo- or heterodimeric cytokine  
receptor.
- 10 3. A modified protein according to claims 1 or 2 wherein at least one molecule  
segment which contributes to a disordered structure is deleted.
4. A modified protein according to claim 3 truncated in at least one terminal end.
- 15 5. A modified protein according to claim 4 truncated in its C-terminal end and in its  
N-terminal end.
6. A modified protein according to claim 5 being human growth hormone receptor  
(hGHR).
- 20 7. A modified human growth hormone receptor (hGHR) according to claim 6 having  
31 or 33 amino acid residues removed in its N-terminal end.
8. A modified human growth hormone receptor (hGHR) according to claim 6 or 7  
25 having 3 or 4 amino acid residues removed in its C-terminal end.
9. A modified human growth hormone receptor (hGHR) according to any of claims 6  
to 8 consisting of residues 32-237, 32-234 or 34-233 of the native molecule.
- 30 10. A modified human growth hormone receptor (hGHR) according to claim 9  
consisting residues 32-237 of the native molecule.

11. Crystals of a receptor protein according to any of claims 1 to 10 to any of claims 1-10 suitable for binding studies with ligand candidates.
- 5    12. Crystals according to claim 11, wherein the contact surface between two molecules is between 200 to 1800 Å<sup>2</sup> (square ångström) and more preferably between 100 to 900 Å<sup>2</sup> (square ångström).
- 10    13. Crystals according to claim 11 or 12 containing at least 50 % (v/v) of a solvent acceptable for binding studies.
14. Crystals according to claim 13 containing about 60 to 80 % (v/v) of a solvent.
- 15    15. Crystals according to any of claims 11 to 14 capable of being frozen with gaseous or liquid nitrogen with maintained capacity of diffraction to at least 3.5 Å by using synchrotron radiation source.
- 20    16. Crystals according to claim 15 capable of being frozen with gaseous or liquid nitrogen with maintained capacity of diffraction to at least 3.5 Å by using synchrotron radiation source.
- 25    17. Crystals according to any of claims 11 to 16 capable of being resistant to an addition of up to 10% (v/v) of DMSO (dimethylsulfoxide) and up to 5 % (v/v) of DMF (dimethylfluoride) for at least 24 hours.
18. Crystals according to any of claims 11 to 17 **characterized in that** they are formed at pH between 5.0 to 8.5.
- 30    19. Crystals according to claim 18 **characterized in that** they are formed at a pH between 7.0 and 8.0.

20. Crystals according to any of claims 11 to 17 formed in the presence of one or more salts having a concentration between 0.15 M and 1.0 M.
21. Crystals according to claim 20, wherein the salt(s) is(are) selected from a group consisting of ammonium sulfate, lithium sulfate, sodium phosphate, potassium phosphate, sodium chloride, lithium chloride, ammonium acetate, sodium acetate, magnesium chloride, sodium formate and sodium citrate.
22. A method of designing drugs with cytokine receptor activity by employing the crystals according to any of claims 11 to 21 in binding studies with selected ligand candidates.
23. A method according to claim 22 involving dimerization of the receptor.
24. A method according to claims 22 or 23, wherein the crystals are soaked or co-crystallized with a solution comprising the ligands.
25. A method according to any claims 22 to 24, wherein the receptor is a modified growth hormone receptor investigated with ligands having potential growth hormone activity.
26. A method of obtaining improved cytokine receptor crystals involving the subsequent steps of:
- (i) solving the receptor three-dimensional structure complexed to a ligand by crystallographic methods,
  - (ii) identifying regions of the receptor molecule which may contribute to disorder in a crystalline state,
  - (iii) producing modified receptor molecules without said regions, and
  - (iv) crystallizing the modified receptor without the presence of a ligand.
27. A method according to claim 26 involving the extracellular part of the receptor.

28. A method according to claim 26 or 27, wherein said receptor is human growth hormone receptor.
- 5      29. A method according to claim 28, wherein said ligand is human growth hormone.

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Fig. 1

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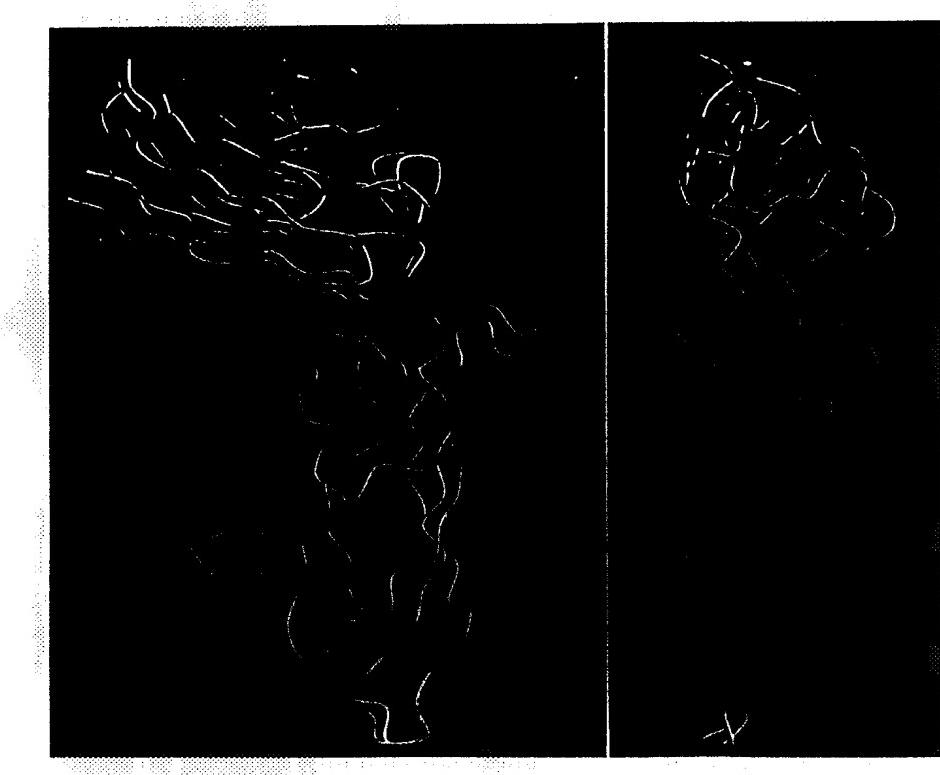


Fig. 2

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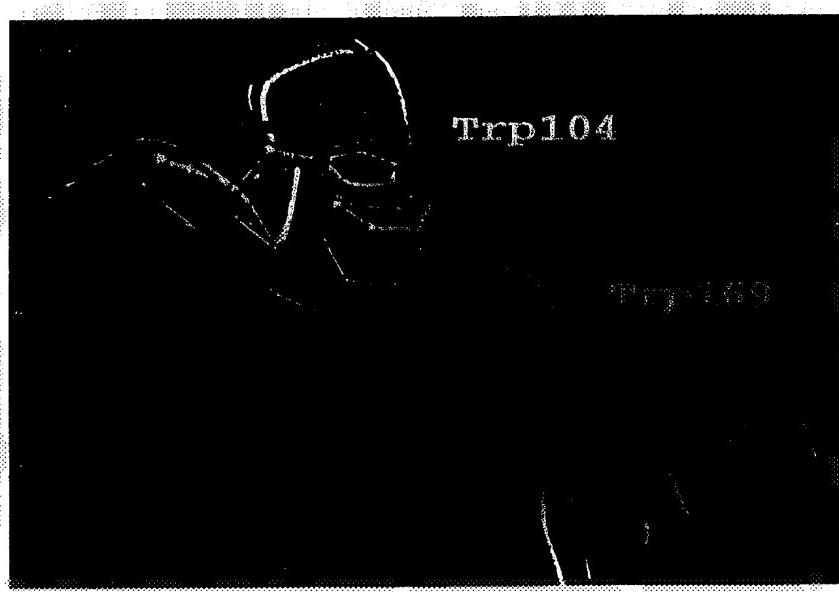


Fig. 3

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/00277

## A. CLASSIFICATION OF SUBJECT MATTER

**IPC6: C07K 14/715 // C07K 1/30**

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**IPC6: C07K**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

**SE,DK,FI,NO classes as above**

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JBC - Sundström et al, Volume 271, No 50, December 1996, Michael Sundström et al, "Crystal Structure of an Antagonist Mutant of Human Growth Hormone, G120R, in Complex with Its Receptor at 2.9 Å Resolution", page 32197 - page 32203, discussion third paragraph, and result	1-6,8,11-29
A	third paragraph --	7,9-10
A	Science, Volume 255, January 1992, Abraham M. De Vos et al, "Human Growth Hormone and Extracellular Domain of Its Receptor: Crystal Structure of the Complex" page 306 - page 312 --	1-29

Further documents are listed in the continuation of Box C.  See patent family annex.

\* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

26 May 1998

Date of mailing of the international search report

08-06- 1998

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/00277

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WPI accession no. 94-238767, TANPAKU KOGAKU      KENKYUSHO KK: "Extracellular C-domain protein      of growth hormone receptor (hGHR-CD) - is used      to enhance growth hormone function";      &amp; JP,A,6172394</p> <p style="text-align: center;">-- -----</p>	1-10